Induction of Post-Irradiation Conditioned Avoidance Behavior by Intraperitoneal Injection of Brain Tissues[1]

The effect of X-irradiation on conditioned avoidance behavior has been the subject of many investigations during recent years. Although the volume of results published was quite impressive, the mechanism of the observed phenomenon is still unanswered. Often, saccharin sodium and sometimes sucrose and sodium chloride have been used as conditioned stimuli with ionizing radiation as unconditioned stimulus. The animals used in experiments were exposed directly to radiation after a taste preference was established[2-10]. Some recent studies carried out in our laboratories indicated that direct exposure to radiation is not necessary for inducing avoidance behavior. Mice, after showing definite preference to saccharin or sucrose, avoid them after these sweet solutions are exposed to relatively low doses of X-irradiation. The same results are also observable when very small amounts of H_2O_2 are added to the solutions[11]. While many questions were still unanswered regarding various aspects of post-irradiation avoidance behavior, we have conducted a series of pilot experiments and observed an extremely interesting result: The transferability of avoidance behavior by transferring brain substance. Follow-up experimentations have confirmed the following observations: 1. Post-irradiation avoidance behavior can be induced by transplantation of brain tissues[12]. 2. Post-irradiation avoidance behavior can be induced by i.p. injection of brain tissues.

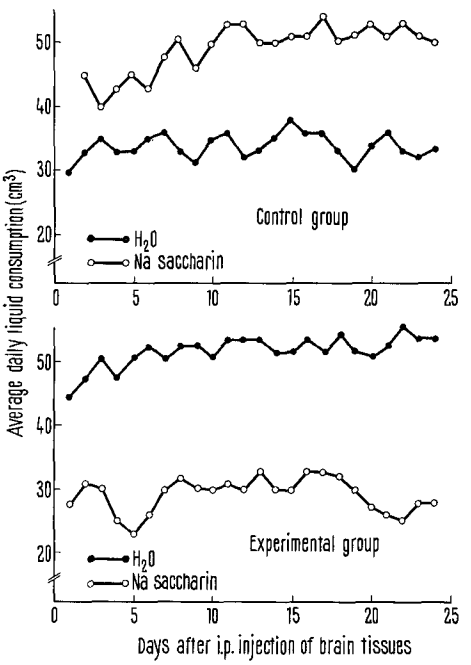
This paper deals only with the latter observation. Still under studies is the possible transfer of post-irradiation avoidance behavior by oral administration of brain tissue as well as the transferability of this behavior by using substance from parts of the body other than the brain.

Method. Male CF_1 mice, 50–60 days old, were used as experimental subjects. The animals receiving brain substance were divided randomly into groups of 30 mice each according to the arrangement in the Table. Thus, 180 recipient animals were involved in each of the 3 experimental runs. After an adaption period for about 1 week, the mice that served as donors were conditioned for saccharin preference. When this preference of the sweet solution (1% per weight) was definitely established, a 24 h (all) liquid deprivation took place before the animals were exposed to sham or X-irradiation (400 R). There were 10 animals in each donor group. Radiation was provided by a 400 kV, 5 mA Maxima 400 therapy unit (HVL 2.53 mm Cu). The average dose rate was about 80 R/min. Within 30 min after sham or actual X-irradiation, all donor animals were sacrificed by spinal cord severance in the cervical region. Brains to be used for control and experimental groups were ground up separately in physiological Ringer solution (5 ml/brain) in sterile mortars at average temperature of about 40 °C until fairly homogenous tissue suspensions were achieved. Each solution was then injected into the peritoneal cavity (1 cm^3) of the recipient animals.

Results and discussion. During the taste preference test, the difference in average daily saccharin and water consumption was between 15–20 cm^3 in favor of the sweet solution. After the injection of brain tissues from the donors, the trend was still clearly observable in the 5 control groups (Table). Since there was no significant difference between the data obtained from 5 control groups, only control group V was chosen for comparison with the experimental group. Thus, the different factor between these 2 groups was that the experimental group was

Conditions of the donors

Naive recipient group	Saccharin preference conditioned	Radiation exposure	Sham exposure
Control I	No	No	Yes
Control II	No	Yes	No
Control III	No	No	No
Control IV	Yes	No	No
Control V	Yes	No	Yes
Experimental	Yes	Yes	No



Transferability of post-irradiation avoidance behaviour by i.p. injection of brain tissues.

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actually exposed to 400 R of X-irradiation while the control group received only sham exposure. The Figure shows the average daily liquid consumption of these 2 groups. From the upper graph one observes a difference of about 35% between daily water and saccharin consumption. The animals in this group definitely prefer sodium saccharin solution to plain tap water as usually observed. In the lower graphs, however, this trend is just reversed. The average daily water consumption exceeds that of sodium saccharin almost 2 to 1.

SCARBOROUGH and MCLAURIN[13] have reported that saccharin solutions, when injected i.p. prior to radiation exposure, does not produce a conditioned aversion. Neither have we observed any avoidance when H_2O_2, a confirmed radiolytic product of irradiated solutions, was injected into the peritoneal cavity of the animals. Since over a decade ago when the first investigators reported that information could be transferred, a number of experiments have been performed but a conclusive demonstration is still yet to be presented. New models were offered to explain the possible mechanism of 'information' or 'learning' transfer, ranging from the idea that neurons may have high chemical specificity[14] to electron-microscope evidence of the existence of intrasynaptic protein filament[15].

The learning and/or behavioral aspect of post-irradiation aversion in mice is still being questioned. Whether the effect of irradiation really changes the composition of the brain in the animals trained to prefer sodium saccharin is a difficult question to answer. P^{32}-labeled RNA, according to LUTTGES et al.[16], was not traceable in the brain after i.p. injection; and nucleic acid extracted from the brains of trained animals does not produce 'transfer of learning' when injected i.p. or intraventricularly. REINIS[17], however, found significant 'transfer effect' by i.p. injection of brain extracts. As we mentioned earlier, we are still very far from being in a position where we can offer any acceptable hypothesis.

Zusammenfassung. Man kann «post-irradiation»-bedingtes Ablehnungsverhalten indirekt herbeifuhren, indem man Hirngewebe von abgerichteten Mäusen in den Peritonealraum der unabgerichteten Mause injiziert. Die «Empfänger» verhalten sich, als seien sie selbst dem Saccharinbevorzugungs-Test und anschliessend ionisierender Bestrahlung ausgesetzt worden. Wir haben zur Zeit noch keine stichhaltige Erklärung für diese interessante Beobachtung.

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Effects of Ergocornine and 2-Br-α-Ergokryptin (CB-154) on the Formation of Mammary Hyperplastic Alveolar Nodules and the Pituitary Prolactin Levels in Mice

Several investigations have demonstrated that ergocornine suppresses deciduoma formation[1] and implantation[1], terminates pseudopregnancy[2], early pregnancy[2] and lactation[3], inhibits the growth of prolactin responsive carcinogen-induced mammary tumor[4] and causes endogenous estrogen-progesterone imbalance[5]. The other ergot alkaloids, α-ergokryptin and 2-Br-α-ergokryptin (CB-154), were also reported to inhibit fertility and lactation in rat[6]. SHELESNYAK[7] found that the pregnancy was protected against ergocornine interference by the additional treatment of prolactin. All these results infer that these ergot alkaloids would inhibit the anterior pituitary secretion.

It is well known that hyperplastic alveolar nodules (HAN) of the mammary gland in mice represent the preneoplastic state in mammary tumorigenesis and that prolactin has a prominent role in the formation of HAN[8]. The present experiment was carried out in order to investigate whether or not ergocornine and CB-154 suppress the formation of HAN, and the pituitary prolactin levels of these ergot alkaloids treated mice were also determined.

Materials and methods. Animals used were 7- to 8-month-old multiparous female mice of C3H/He strain more than 1 month after the last lactation. They were divided into 3 groups consisting of 10–11 mice each. Groups I and II were given s.c. injections of 0.2 mg of ergocornine methanesulfonate and CB-154 suspended in 0.1 ml of physiological saline daily for 20–23 days, respectively. Group III received no treatment and was served as control. All mice were examined by vaginal smears once a day throughout the experiment, beginning 10 days before the start of injections. They were killed by decapitation at 20–23 days after the start of injections when they showed the proestrous to estrous smears. The anterior pituitary was immediately removed, weighed and kept at −20 °C until assayed. Prolactin and growth hormone (GH) levels in the anterior pituitary were determined by recently developed disc electrophoretic method

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